RESEARCH ARTICLE

Deformation Behaviour of 3D Printed Reinforced Hydroxyapatite/Gelatin Bio-Ceramics

A.Megalingam[1,*], Rejikumar Rajamony[2], M. A.Perumal[3], S.Malliga[4]

[1] Faculty of Mechanical and Automotive Engineering Technology,
Universiti Malaysia Pahang Al-Sultan Abdullah, 26600 Pekan, Pahang, Malaysia
[2]Faculty of Institute of Sustainable Energy, Universiti Tenaga National,
Jalan Ikram-Uniten, Kajang, 43000, Selangor, Malaysia
[3]Vijayam Institute of Engineering and Technology, Nagercoil, 629001, Tamil Nadu, India
[4]Vivekananda College, Agastheeswaram, Kanyakumari, 629004, Tamil Nadu, India

ABSTRACT: This paper investigates the deformation behaviour of three-dimensional (3D) printed reinforced hydroxyapatite/gelatine bio-ceramics. The worldwide demand for organ replacement or tissue regeneration is increasing steadily. The advancements in tissue engineering and regenerative medicine have made it possible to regenerate such damaged organs or tissues into functional organs or tissues with the help of 3D bioprinting. In this technology, 3D bioprinting is a rapid prototyping technique that excels at producing quality products using complex shapes and materials that are difficult to handle with precise parameters. Bio-ceramic-based composites are increasingly preferred for bone tissue preparation due to their desirable osteogenic properties. Although the bioactivity and low toxicity of hydroxyapatite are considered favourable, their poor mechanical properties, low fracture toughness, and tensile strength are of concern. The use of graphene oxide (GO) as reinforcement, has the potential to improve the mechanical and biological properties of bio-ceramic composites. Cross-linking by genipin adds additional strength to the model. However, there is a lack of deformation behaviour defining mechanical properties in the literature. Thus, there is a lack of comprehensive understanding of such bio-ceramics. The study is expected to contribute to ongoing research on biocompatible materials for the development of patient-specific implants and scaffolds by closely studying their deformation behaviour. It presents a recommendation for the use of GO in the formulation of bio-ceramic composites and its cross-linking by genipin to improve mechanical properties.

KEYWORDS: Deformation Behaviour, Extrusion-based 3D Printing, Hydroxyapatite/ Gelatin, Bio-ceramics, Graphene Oxide, Genipin.

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1.0 INTRODUCTION

Tissue engineering is an advanced technology in modern medicine that reflects the dynamic convergence of principles from engineering, biology, and materials science. It has made significant strides in regenerative medicine, with applications ranging from tissue repair to disease modelling [1]. While numerous challenges persist, innovative approaches, interdisciplinary collaboration, and ongoing research hold the potential to transform tissue engineering into a cornerstone of modern medicine, offering hope to countless patients in need of functional tissue replacements [2]. Robert Langer's study outlines the basic elements of tissue engineering, emphasizing the critical role of scaffolds, cells, and biomaterials in the development of engineered tissues [3].

Bio-ceramics play a pivotal role in the biomedical field, offering a versatile range of applications that harness their exceptional biocompatibility and bioactivity. Bio-ceramics, characterized by their biocompatibility, mechanical properties, and ability to bond with living tissues, have emerged as vital components in the development of medical devices, implants, and tissue engineering scaffolds [4]. The diversity of bio-ceramic applications that include load-bearing orthopaedic implants such as hip and knee replacements, tooth restoration, bone graft replacement, and biodegradable scaffolds for tissue engineering. Wong's study presents the

**Corresponding Author: megalingammahesh@gmail.com*
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challenges associated with bio-ceramic materials, achieving optimal mechanical properties while maintaining biocompatibility, addressing issues related to long-term stability and deformation, and improving the integration of implants with host tissue [5]. According to previous studies on the mechanical properties of hydroxyapatite/gelatin composites, approximately Young's modulus (elastic modulus) values are 1 to 10 GPa, tensile strength values are 10 to 40 MPa, fatigue strength values are 5 to 20 MPa, compressive strength values are 30 to 200 MPa, and hardness values are 30 to 200 MPa. Values are 20 to 100 HV. The addition of graphene to hydroxyapatite/gelatin composites improves tensile strength by 20 to 50%, Young's modulus (elastic modulus) by 30to 70%, fracture toughness by 10 to 40%, compressive strength by 20 to 60%, hardness by 20% Studies show it can improve up to 50% [6].

The development of biomaterials represents a dynamic and rapidly evolving field at the intersection of materials science, biology, and medicine. Researchers in this field focus on creating materials with properties such as biocompatibility, bioactivity, and mechanical strength, tailored to meet the demands of various medical applications. The quest for biomaterial innovation spans a broad spectrum, including the development of synthetic materials, natural polymers, and hybrid constructs that mimic the complex properties of biological tissues. Advancements in biomaterial science have played a pivotal role in medical implants, drug delivery systems, tissue engineering, and regenerative medicine [7]. The importance of 3D-printed models in the development of biomaterials cannot be overstated, as this technology has emerged as a transformative tool in advancing the field. 3D printing allows for the precise fabrication of complex structures with intricate geometries, enabling researchers to create customized scaffolds, implants, and tissue constructs [8].The ability to rapidly prototype and redesign designs through 3D printing speeds up the development process, and 3D-printed models play an important role in preoperative planning. The scope of application for 3D-printed models in the development of biomaterials for the advancement of medical science and healthcare is broad and diverse.3D-printed models serve as invaluable tools for evaluating and refining biomaterial designs, ensuring optimal biocompatibility, structural integrity, and functionality.

3D printing is an attractive technology, also known as additive manufacturing (AM), that creates three-dimensional objects layer by layer from digital designs. The integration of bio-ceramics and 3D printing has revolutionized biomaterials and tissue engineering. The fusion of bio-ceramics and 3D printing technology has shown to be a significant advancement in the field of biomedical materials [9]. As explored in depth in the literature; this integration provides a transformative approach to the development of patient-specific, biocompatible, and structurally complex biomaterials. Lin's [10] comprehensive literature review further delves into the extensive applications of bio-ceramics in conjunction with 3D printing. In orthopedics, the combination of these technologies has paved the way for patient-specific implants that precisely replicate the complex anatomy of bones and joints, reducing the risk of complications and improving patient outcomes. In regenerative medicine and tissue engineering, 3D-printed bio-ceramic scaffolds provide a nurturing environment for cell growth and tissue regeneration, offering hope for the development of functional organs and tissues. Moreover, the review examines how this innovative synergy extends to dental restorations, craniofacial reconstruction, and even drug delivery systems, demonstrating the far-reaching impact of 3D-printed bio-ceramics in the biomedical field. Osouli-Bostanaba's study provided a valuable source of information, stimulating interest in exploring the opportunities, challenges, and current developments in this dynamic and growing biomaterials and healthcare area [11].

Graphene oxide, a derivative of graphene, has garnered widespread attention due to its versatile properties and myriad applications across various scientific and technological domains. Graphene oxide acts as a reinforcing agent in composite materials due to its unique two-dimensional structure, mechanical strength, and thermal stability [12]. Improving the mechanical strength of 3D-printed hydroxyapatite/gelatin bio-ceramics with graphene oxide is attracting attention as various studies are gaining importance. Hydroxyapatite and gelatin, while biocompatible, often exhibit mechanical weaknesses that hinder their suitability for load-bearing applications in bone tissue engineering. The incorporation of

graphene oxide, known for its exceptional mechanical properties and biocompatibility, holds great promise in reinforcing the structural integrity of these bio-ceramics [13]. The unique combination of hydroxyapatite and gelatin with graphene oxide aims to capitalize on the strength and flexibility of graphene, resulting in a composite material that not only mimics the natural properties of bone but also offers improved mechanical strength. This innovative approach reflects a commitment to advancing biomaterials for regenerative medicine, paving the way for more robust and effective solutions, particularly in bone tissue engineering and orthopedic applications.

The deformation behaviour of bio-ceramics is in demand in the development of advanced biomaterials for various biomedical applications [14]. Understanding how these composite materials respond to mechanical stresses, such as compression, tension, or bending, is essential for ensuring their structural integrity and functional performance within the human body [15]. There is a lack of research on the effects of reinforcement with GO and its cross-linking to improve the mechanical properties of hydroxyapatite/gelatinebio-ceramics. There is a gap in the deformation behaviour of samples with cross-linked and without cross-linked by genipin at different reinforcement ratios, which this study is expected to fill. This study aims to present in detail the preliminary deformation behaviour of hydroxyapatite/gelatinbio-ceramics reinforced with 3D-printed graphene. It will lead to improved designs and improved performance in tissue engineering, orthopedic implants, and other biomedical devices.

2.0 MATERIALS AND METHODS

2.1 Materials

Hydroxyapatite (HA) Powder (57.4nm), Type A Gelatin biopolymer (50000 Mw), Graphene powder, Sulfuric acid (H_2SO_4), Potassium permanganate (KMnO4), and Genepin ($C_{11}H_{14}O_5$) were used in this experimental study. All chemicals are purchased from MP Biomedicals Korea, Seoul, South Korea.

2.1 Sample Preparation

Hummer's method was used to produce graphene oxide (GO) by oxidation.One gram of graphite powder was added to 23 ml of concentrated H_2SO_4 solution and stirred for twohours with a mechanical stirrer. 3 g of $KMnO_4$was added little by little to this mixture.

The temperature was increased from 20 to 40◦C, after stirring for 30 min heated at 70 ◦C for 45 min, then 3 mL of DI water was added and heated at 100◦C for 5 min. Also, 40 ml of distilled water is added and heated for 15 minutes. Finally, 140 mL of DI water and 3 mL of 30% H_2O_2 were added to stop the reaction. After that, the filtered solution reacted with 10% HCl and distilled water. By-products are thereby removed. The solution was dried in an oven at 40◦C for 24 hours.

The synthesized GO powder was dispersed in distilled water by ultrasonication in a bath sonicator, after successfully dispersing the GO in the water, added the gelatin powder to the GO solution. Maintain 20% w/v concentration. Gelatin was dissolved in the mixture using a magnetic stirrer at 70 °C for 2 h. HA powder is added to this solution and mixed for 1 minute only. It contains a mixture of 70% HA, 20% Gelatin, and 10% GO.

Genipin was used to reinforce the 3D-printed model. For this, genipin powder at a concentration of 0.5%w/v was dissolved in a phosphate-buffered saline (PBS) solution. The sample is immersed in this genipin solution. Air bubbles in the bio-ceramics are removed by gently shaking the container. The solution was heated to 35◦C and maintained constant. After 24 hours the sample was taken out and the silk was soaked in PBS to neutralize the genipin. The prepared sample followed the previous study preparation method [15,16].

2.3 Extrusion-based 3D Printing Machine

An extrusion-based 3D printing machine was used to print the sample in three dimensions. In this setup, a nozzle tip diameter of 500µm has been selected, determining the size and precision of the extruded filament. The bed temperature, set at 35 degrees Celsius, creates an ideal surface for initial layer adhesion, promoting successful printing. Meanwhile, the nozzle temperature is carefully maintained at 50 degrees Celsius, ensuring that the thermoplastic filament reaches its optimal melting point for extrusion. A schematic diagram of the extrusion-based 3-D printing machine used for this study is presented in Fig. 1.

The printing speed, configured at 10 mm/s, strikes a balance between print quality and speed, allowing for precise layer deposition

while avoiding potential issues such as overheating or filament flow inconsistencies. Lastly, the pressure control at 500 kPa regulates the extrusion force, guaranteeing a consistent flow of material through the nozzle. Finally, the printed material is reinforced to improve its mechanical and biocompatible properties. Genipin is used to reinforce the 3D-printed model. For this, genipin powder at a concentration of 0.5%w/v was dissolved in a phosphate-buffered saline (PBS) solution. The sample is immersed in this genipin solution. Air bubbles in the bioceramics are removed by gently shaking the container. The solution was heated to 35°C and maintained constant. After 24 hours the sample was taken out and the silk was soaked in PBS to neutralize the genipin. Specification and processing parameters for an extrusion-based 3D printing machine are presented in Table.1.

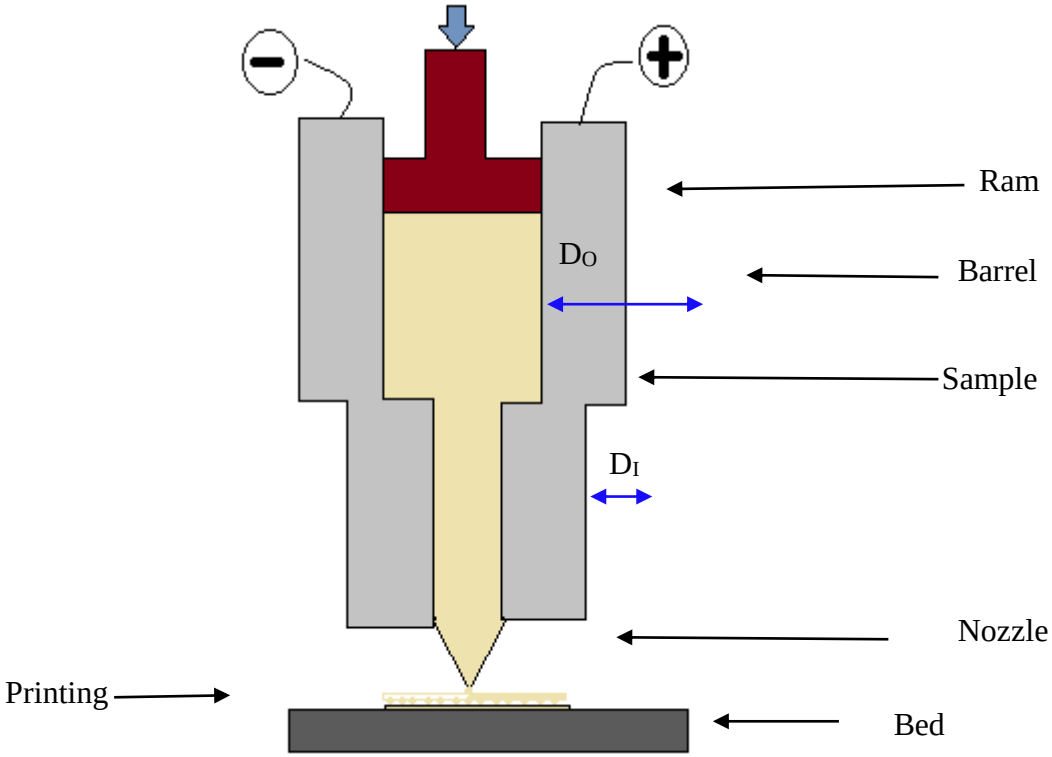

Fig. 1 Schematic diagram of extrusion-based 3-D printing machine [20]

Table 1: Specification and processing parameters for an extrusion-based 3D printing machin

NO	PARAMETERS	VALUES
1	Nozzle Inner Diameter	510µm
2	Layer thickness	0.1 mm
3	First layer	0.3 mm
4	Solid Layer	12
5	Nozzle Outer diameter	0.3 mm
6	Filament diameter	1.5 mm
7	Bed temperature	35

8	Nozzle temperature	50◦C
9	Printing Speed	10 mm/s
10	Pressure	500 kpa
11	Quenching period	24 Hrs
12	Humidity	70 to 80%
13	Room temperature	20 to 30 ◦C

The same processing parameters were followed for both compressive and tensile testing in this study. The processing parameters are given in Table 2 .Samples 1 to 6 are cross-linked samples by genipin and samples 4 to 6 are non-cross-linked samples. To cross-link by Genipin, the genipin powder at a concentration of 0.5% w/v was dissolved in a phosphate-buffered saline (PBS) solution. The sample was immersed in this genipin solution. Air bubbles were then removed by shaking the container gently to remove them. The solution was heated to 35 °C and maintained constant. A sample is taken after 24 hours. Genipin was neutralized by soaking in undiluted PBS.

Table 2: Processing parameters for compression and tensile test

Sample Number	Graphene Oxide(GO)	Cross-linked(CL)
1	0%	With Genepin
2	0.5%	With Genepin
3	1.0%	With Genepin
4	0%	-
5	0.5%	-
6	1.0%	-

2.4 Compression Test

Cylindrical specimens of 26 mm diameter and 52 mm height were used for this compression test (ASTM D 5024-95 standard).A Shimadzu universal testing machineAG-25TA (Japan) is used for this experiment, the photo view and schematic working view are presented in Fig.2. Pre-loading procedures were carried out to condition the machine before testing. It operates at a constant cross-head speed of 0.5 mm/min. Processing parameters for the compression tests are presented in Table.2

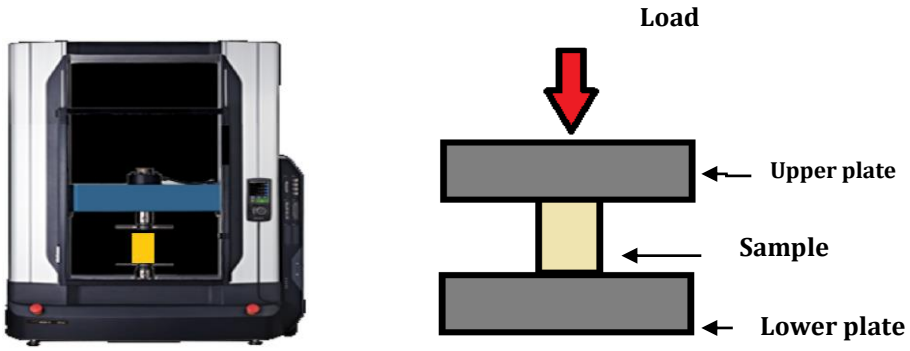

Fig. 2 Universal testing machine (a) Illustration view (b) Schematic working view [18]

2.5 Tensile Test

A tensile test was performed based on ASTM D638 Type 5. Testing was performed at room temperature using an Instron IX material testing machine. It operates at a constant cross-head speed of 2 mm/min.Tensile strength and elastic modulus values were obtained from five samples at each parameter. ASTM D638 type 5 tensile specimen shape is presented in Fig 3. Processing parameters for tensile testing follow **Error! Reference source not found.**. All parameters are mm in Fig 3.

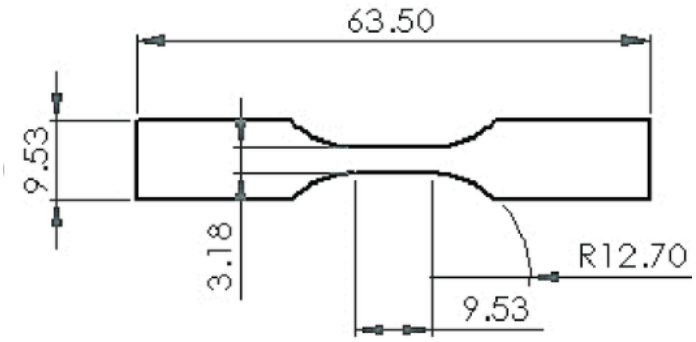

Fig. 3 Tensile specimen (ASTM D638 Type-5) [19]

3.0 RESULTS AND DISCUSSION

3.1 Compression Analysis

The investigation into the compressive test behaviour of 3D printed reinforced hydroxyapatite/gelatinbio ceramics revealed notable variations in compressive strength under different conditions. A bar chart of the values obtained in the compressive strength test results of the 3D-printed reinforced hydroxyapatite/gelatine bioceramics is presented in Fig.4.

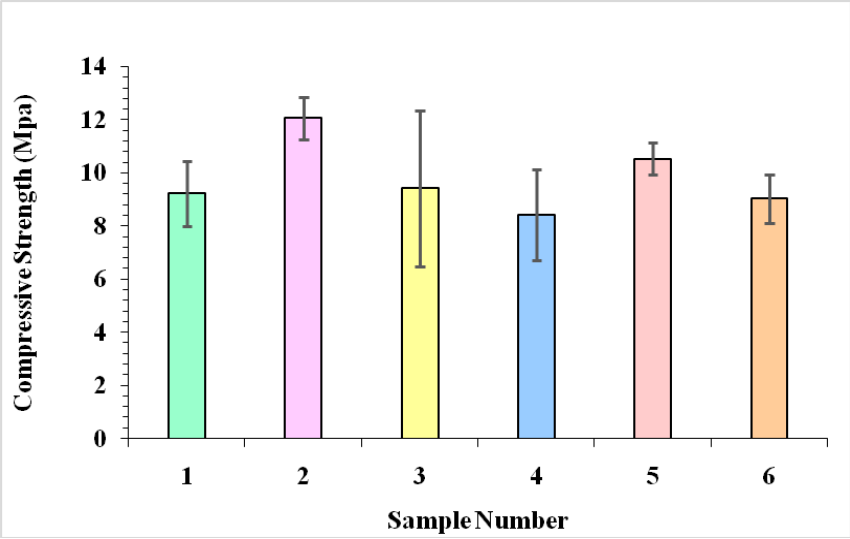

Fig. 3 Bar chart of compressive strength test results of 3D printed reinforced hydroxyapatite/gelatin bio-ceramics

Sample 1: Unreinforced and crosslinked (CL)., Sample 2: 0.5 % GO, and with CL., Sample 3: 1% GO and CL.,Sample 4: Unreinforced and without CL., Sample 5: 0.5 % GO,and without CL., Sample 6: 1% GO and without CL.,

Compressive strength was assessed at varying concentrations of graphene oxide, including 0%, 0.5%, and 1%, and tested on two different crosslinked with and without graphene conditions. Among the samples cross-linked by genepin, the bio-ceramics were found to have the lowest compressive strength (9.21MPa) in the absence of graphene oxide (0%). However, as the concentration of graphene oxide was increased to 0.5%, a significant improvement in compressive strength was observed. This concentration gave significant high compressive

strength (12.06 MPa). In contrast, at 1% graphene oxide concentration, the compressive strength was medium (9.41 MPa). Among the prepared samples, the GO-reinforced hydroxyapatite/gelatin bio-ceramics sample showed remarkable properties as expected. A previous study reported that the compressive strength values of 3D printed samples on hydroxyapatite/gelatin bio-ceramics, not cross-linked by Genipin, ranged from 8.94 ±1.24 to 6.48 ±1.61 MPa in 1% GO and 10.28 ± 1.08 MPa in 0.5% GO. These results were similar to those of the previous study [17,20]. All values in samples not cross-linked by graphene revealed lower compressive strength than cross-linked samples. The compressive strength of unreinforced sample number 1 with graphene oxide was 8.43MPa, whereas the compressive strength of sample 2 reinforced with 0.5% graphene oxide was 10.53MPa. The compressive strength of 1 % reinforced sample number 3 showed 9.03 MPa which was lower than that of the 0.5 % reinforced sample. The lack of reinforcement at 0% graphene oxide concentration resulted in a weak structure that was less able to withstand compressive forces. The addition of GO to hydroxyapatite /gelatin bioceramics increased their compressive strength through a synergistic effect. This prevented crack propagation and increased the overall material stiffness, thereby strengthening the structure of the bio-ceramic matrix [20].The ability of GO to form strong hydrogen bonds with gelatin [21]. This result underlines the beneficial effect of graphene oxide reinforcement on the material's ability to resist shrinkage. Also, at all tested concentrations of graphene oxide, a trend towards decreased compressive strength was observed with genipin. The compressive strength of the specimens unbonded by Genipin was found to be about 10% lower. A bar chart of the values obtained in the compressive toughness test results of the 3D-printed reinforced hydroxyapatite/gelatin bio-ceramics is presented in Fig. 5.

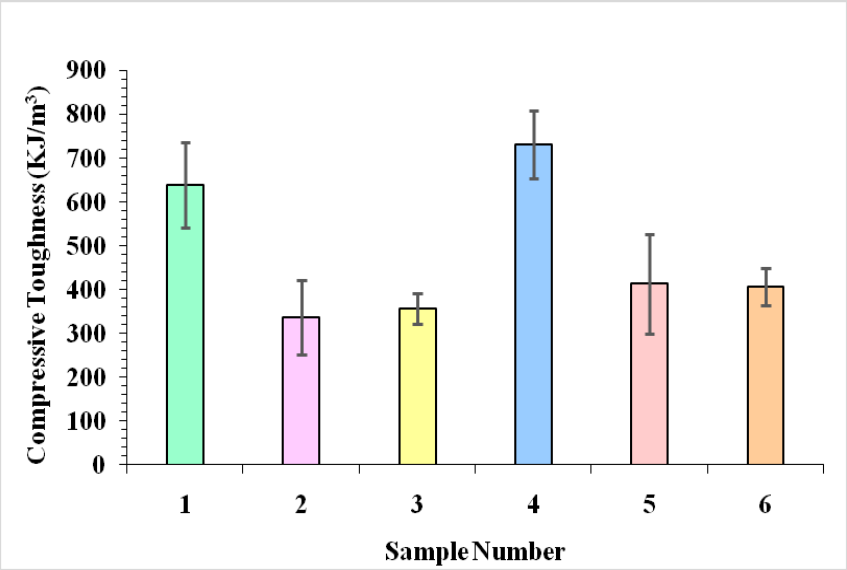

Fig. 4 Bar chart of compressive toughness test results of 3D printed reinforced hydroxyapatite/gelatin bio-ceramics

Sample 1: Unreinforced and crosslinked (CL)., Sample 2: 0.5 % GO, and with CL.,
Sample 3: 1% GO and CL., Sample 4: Unreinforced and without CL.,
Sample 5: 0.5 % GO,and without CL., Sample 6: 1% GO and without CL.

The lower compressive toughness observed in the absence of graphene oxide (0% concentration) was consistent with expectations (638KJ/m^3), as graphene oxide is commonly employed as a reinforcement agent to enhance the mechanical properties of composite materials. Its absence resulted in a less resilient structure, making it more susceptible to fracture and failure under compressive loads. The compressive toughness observed at 0.5% graphene oxide concentration was 336.66 KJ/m^3, which shows a highly beneficial effect on the energy-absorbing capacity of the reinforcement and resistance to fracture during compression. This concentration level led to a high compressive toughness, indicative of improved fracture resistance. A significant trend emerged in the

non-cross-linked samples, with increased compressive hardness at all tested concentrations of graphene oxide. This material reduces its ability to withstand high-pressure loads.

3.2 Tensile Analysis

The results obtained from the tensile test and elongation percentage study of 3D-printed reinforced hydroxyapatite/gelatinbio-ceramics are presented in Fig.6, and Fig.7. At different concentrations of graphene oxide (0%, 0.5%, and 1%), and the effects on samples cross-linked and uncross-linked by genipin were illustrated.

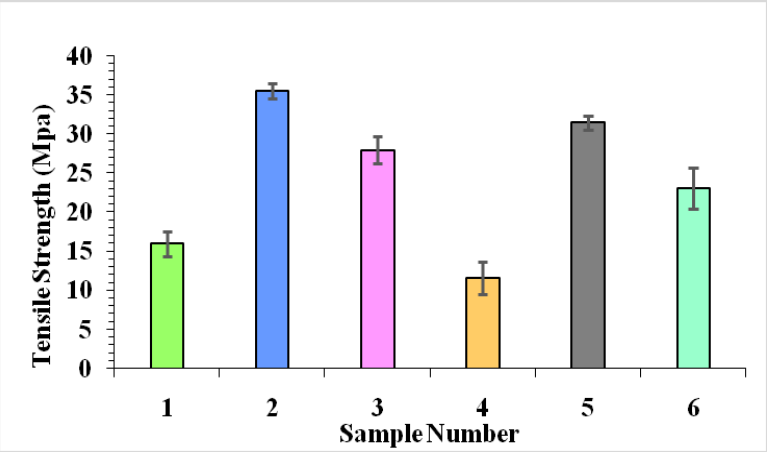

Fig.6 Bar chart of tensile strength test results of 3D printed reinforced hydroxyapatite/gelatin bio-ceramics

Sample 1: Unreinforced and crosslinked (CL)., Sample 2: 0.5 % GO, and with CL., Sample 3: 1% GO and CL., Sample 4: Unreinforced and without CL., Sample 5: 0.5 % GO,and without CL., Sample 6: 1% GO and without CL.

It is evident that the concentration of graphene oxide has a discernible impact on the tensile behaviour of the bio-ceramic composites. Among the cross-linked samples of genipin, it was found that it had the lowest tensile strength of 15.96 MPa in the absence of graphene oxide (Sample-1). However, as the concentration of graphene oxide increased to 0.5%, a significant improvement in tensile strength was observed. This concentration gave a significantly higher tensile strength value was 35.53 MPa. Conversely, at 1% graphene oxide concentration, the tensile strength was medium (27.96 MPa). Similarly, the mechanical properties of the nanofiber membranes showed that the tensile strength values ranged from 20 to 25 MPa. These results are similar to those of a previous study [20]. Like the compressive strength value, all the values in the graphene non-cross-linked samples exhibited lower tensile strength than the cross-linked samples. The tensile strength of the bio-ceramics without graphene oxide was 11.56 MPa, 31.46 MPa when the concentration of graphene oxide was 0.5%, and 23.13 MPa when the concentration of graphene oxide was increased to 1%. The lack of reinforcement at 0% graphene oxide concentration resulted in a weak structure with less ability to withstand tensile loads. The results reveal that the cross-linked model outperformed the unlinked samples in terms of tensile strength. It implies that the cross-linking process, likely by enhancing the intermolecular bonds within the material, led to superior mechanical properties. The addition of graphene oxide and cross-linked by genipin are contributed to the improved tensile properties of the samples, increasing the structural integrity of the bio-ceramic composites.

The concentration of graphene oxide has a discernible impact on the tensile behaviour of bio-ceramic composites. Specifically, the 0% concentration yielded lower tensile values, indicating that the absence of graphene oxide led to reduced mechanical strength. The elongation percentage at 0% concentration was 16.43% in cross-linked samples and 21.9% in non-cross-linked samples. The research findings of this study are consistent with previous results [22]. Conversely, the 0.5% and 1% concentrations produced decreased tensile

values. Elongation percentage in cross-linked samples at 0.5% and 1% concentrations was 6.3 %, and 7.63 % respectively. The addition of graphene oxide helps in improved tensile properties. Previous studies have shown that the elongation of samples in unreinforced hydroxyapatite/gelatin bio-ceramics was less than 5%.The results reveal that the cross-linked model outperformed the unlinked samples in terms of tensile strength. This finding implies that the cross-linking process, likely by enhancing the intermolecular bonds within the material, led to superior mechanical properties. These improved results of the cross-link samples appear to have significant implications for the application of 3D-printed reinforcement.

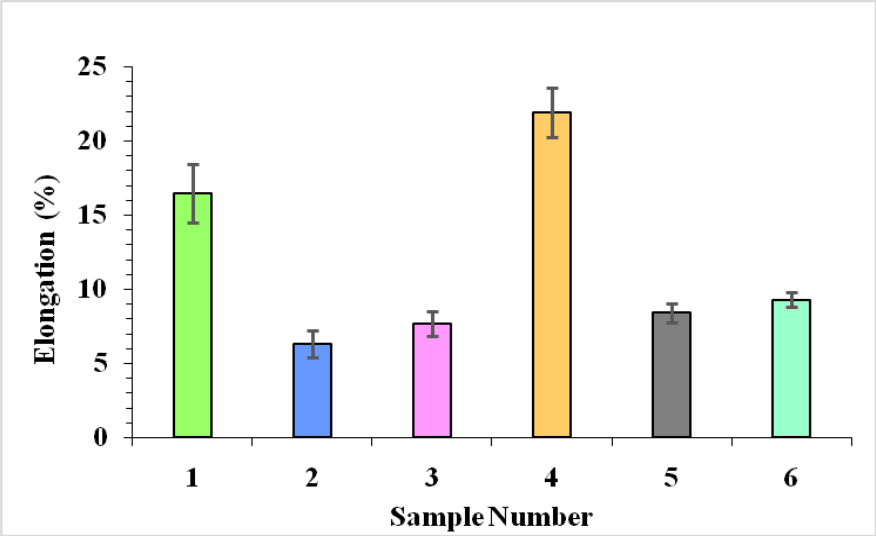

Fig. 5 Bar chart of elongation percentage test results of 3D printed reinforced hydroxyapatite/gelatin bio-ceramics

Sample 1: Unreinforced and crosslinked (CL)., Sample 2: 0.5 % GO, and with CL., Sample 3: 1% GO and CL., Sample 4: Unreinforced and without CL., Sample 5: 0.5 % GO, and without CL., Sample 6: 1% GO and without CL.

CONCLUSIONS

The deformation behaviour of 3D-printedhydroxyapatite/gelatin bioceramics reinforced with graphene oxide was successfully evaluated by both compressive and tensile testing. This study illustrated the implications for cross-linked and non-cross-linked samples by genepin. The compressive strength test results of samples cross-linked by genipin were 9.21 MPa in the absence of graphene oxide, 12.06 MPa when 0.5% graphene oxide was added, and 9.41 MPa at 1% concentration, while the compressive strengths of non-cross-linked samples were 0%, 0.5%, and 1% concentrations were 8.43 MPa, 10.53 MPa, and 9.03 MPa respectively. It has been demonstrated that reinforcement of 3D printed hydroxyapatite/gelatin bioceramics samples with graphene oxide improves mechanical properties, and cross-linking with genipin achieves an additional benefit of about 10%.The tensile strength test results of samples cross-linked by genipin were 15.96 MPa in the absence of graphene oxide, 35.53 MPa when 0.5% graphene oxide was added, and 27.96 MPa at 1% concentration. The tensile strengths of non-cross-linked samples were 15.96 MPa, 31.46 MPa, and 23.13 MPa at 0%, 0.5%, and 1% concentrations, respectively. This study leads to an understanding of the reinforcement with GO and its cross-linking with genipin to improve the mechanical properties of hydroxyapatite/gelatin bioceramics. Additionally, the ability to respond to mechanical properties is explained. These research findings may lead to improved design and improved performance in tissue engineering, orthopedic implants, and other biomedical devices.

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